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Root Disease of Sugar Cane in Louisiana

HISTORICAL.

The root disease of sugar cane was first described from Java in 1895 by Dr. J. H. Wakker, who gave it the name "Dongellanziekten," the disease of the underground part of the cane stalk. He observed the disease on the sprouting cuttings of the "nurseries" and the older canes of the fields; and he found the causative organism to be a fungus which he described and proposed as a new species, *Marasmius sacchari*. This fungus, primarily a saprophyte, has acquired the habits of a wound parasite, and finds in connection with cane cuttings and cane plants conditions most favorable for its full development and fruiting.

At about the same time studies upon a similar disease were begun in the British West Indies, and were carried on for a number of years by several investigators. An extensive account of the disease, however, was not published until 1903, when Mr. A. Howard gave the results of his studies in Barbadoes. He attributes the disease to Wakker's species of *Marasmius*, and adds materially to the knowledge of the conditions favoring the attacks of the fungus upon the cane plant. Further observations made by Mr. F. A. Stockdale lead to the opinion that probably more than one species of *Marasmius* attack sugar cane in the West Indies.

In 1905 Mr. L. Lewton-Brain published a preliminary account of the disease in the Hawaiian Islands, and in 1906 there followed a very complete account by Dr. N. A. Cobb. According to these writers a certain amount of the root disease occurring in the Hawaiian Islands is caused by a species of *Marasmius* which differs in minor characters from Wakker's species, and is accordingly proposed as a new variety, *Marasmius sacchari* variety *hawaiiensis*. Dr. Cobb also attributes a considerable amount of the root disease in the Hawaiian Islands to a fungus of a very different type, *Ithyphallus coralloides,* a member of the so-called stink-horn group of fungi. He expresses the belief that further study will show that still other fungi, having the same general mode of living, play a part in causing root disease.

APPEARANCES INDICATIVE OF ROOT DISEASE.

Affected stools show very constantly and markedly root systems that are deficient in their development, and that show a considerable percentage of dead roots. Such stools have a weak hold on the ground and may be pulled up with comparative ease; they are also the ones most generally prostrated by storms. The canes of these stools are reduced appreciably in size and weight. The leaf system is reduced; and when the supply of available moisture in the soil falls below normal, symptoms of water starvation are apparent, while other stools maintain a normal appearance. When the drouth conditions become more severe, a large percentage of affected stools die. These effects are caused by the deficiency in root system. They are more pronounced in stubble than in plant cane, for reasons that will be indicated later. The lower leaf sheaths (shuck) of affected canes do not fall away, leaving the older part of the canes clean, as is normally the case, but they adhere closely for some distance, as much as eighteen inches, from the surface of the ground, and can be removed only with difficulty. (Fig. 1.) They are seen to be cemented together by the whitish mycelium of a fungus which has a characteristic moldy odor. Under favorable conditions, which seem to occur with comparative infrequency, the toadstool fructifications of the fungus are to be found in connection with the mycelium.

This fungus which cements the leaves is the organism that causes the destruction of the cane roots, from which result the acute symptoms of disease and the ultimate reduction in the yield. The fungus is primarily a soil-inhabiting organism, and its appearance above ground on the somewhat moist and nutritious leaf sheaths where conditions are favorable for its growth, is rather incidental. The fungus may infest a stool of cane, especially in the plant crop, and cement the sheaths in the characteristic way, and yet not affect the roots or injure the stool appreciably in any way. Such a stool can not strictly be regarded as diseased; and yet with the fungus present it is probable that sooner or later the living tissues will be permeated, and typical root disease induced. On the other hand, even in stools that suffer most from root disease, it is usual to find a certain percentage of the individual canes without indications of the fungus above ground. But with all of this, the matting of the leaf sheaths is a most useful indication of the actual or potential presence of root disease. This matting is not very conspicuous until about the time the cane "begins to joint," about August.

THE CAUSE OF ROOT DISEASE.

It has already been indicated that in other countries several distinct fungi have been found to play a causative role in inducing root disease; and we are led to regard the root disease of sugar cane as one presenting a definite group of symptoms which result from the destruction of the cane roots by the attacks of some one of several fungi which differ much in their taxonomic relations, but have the same general life habits. These fungi are not aggressive parasites; for the most part they flourish on non-living parts of the cane, and only under special conditions and in particular situations can they directly attack living tissues.

In Louisiana, a species of fungus which is regarded as being in all probability *Marasmius plicatus*, Wakker, is found constantly associated with growing cane or with decaying cane parts. The canes on which it occurs show generally, though not invariably, a dwarfed growth, a reduced root system, and, during a period of drouth, are the ones to succumb most readily; canes without the fungus have nowhere been observed to show generally these symptoms. Mycelium of the fungus has been observed in living and dead roots. Repeated inoculations with pure cultures have produced in living young cane plants the characteristic symptoms of root disease. The fungus has been recovered in pure culture from such artificially infected plants.

These facts lead to the conclusion that the species of *Marasmius* found generally on sugar cane in Louisiana, which is regarded as *Marasmius plicatus*, Wakker, must be added to the list of fungi that have the ability to induce the so-called root disease of sugar cane.

Specimens of Louisiana cane showing the characteristic mycelial growth, but not the fructifications, have been submitted to the proper authorities at the experiment stations in Java, Hawaii and Barbadoes. In all cases the reports have been that in so far as could be judged from the material, the appearances were those of root disease.

With regard to two species of fungi belonging to the stinkhorn group, and found in some numbers in the cane fields of the State, definite statements cannot be made at present.

THE FUNGUS.

Marasmius plicatus belongs to the toadstool or mushroom group of fungi. In common with other members of the group, the organism has a vegetative part known as the mycelium, made up of minute branching white threads which ramify through the nutrient substratum. It is this mycelium that is found infesting the dead roots and the matted leaf sheaths of the cane plant. When conditions of moisture and food supply are favorable there develop in connection with the mycelium the fructifications of the fungus. They appear at first as small, ball-like masses of mycelium; these increase in size, and gradually become differentiated into the component parts of the fully developed fruit body. These fructifications are seldom observed since they are formed with comparative rarity, and since they remain fresh for a very short time.

The fruit body (Figs. 2 and 3) has the characteristic toadstool form, with an upper expanded portion, the cap or pileus, and a supporting stalk, the stipe. The pileus is dirty white, becoming somewhat darker with age; it is usually about three-fourths of an inch in diameter, but may attain a size of an inch and one-fourth. When young it is convex, and at maturity is almost flat or perhaps slightly concave. Its surface is smooth. On the under side are the radiating gills which have an even, thin edge, and a straight, radial direction. The long gills extend from the margin to the stem, and are attached to the stalk itself rather than to a prominent ring about the stalk. Other shorter gills extend from the margin just far enough to fill in the angles between the longer gills. The stipe is about equal in length to the diameter of the cap, or in some cases, somewhat less. It usually arises from the side of the leaf sheath, and is somewhat curved so as to bring the cap into a horizontal position. It is normally attached to the cap at its central point, but at times this attachment is somewhat eccentric. The stipe is smooth externally, except at the base, which is downy and also enlarged. The whole fruit cap persists for about a day, and then gradually dries, losing its form, but not undergoing immediate disintegration. When moistened it regains something of its original form. The gills produce upon their surface the spores of the fungus (Fig. 7), which are minute oval bodies of microscopic size. They show a pure white color in mass. Their function is to reproduce the fungus plant. This they do, under suitable conditions, by sending out a small thread which may by its continued growth develop into the mycelium of a new generation of fungus.

This fungus grows saprophytically upon decaying vegetable matter, seemingly showing a rather strict selective preference for parts of cane plants. In the laboratory it grows well upon a wide range of nutrient media. In addition to this saprophytic mode of living the fungus has the ability to attack living tissues, probably only when their vigor is impaired, and thus to adopt the mode of life of a parasite. It is in this role that the fungus becomes of economic importance in causing damage to cane plants.

MODES OF DISTRIBUTION.

The spores of fungi are the parts specially formed and adapted for the reproduction and dissemination of the parent organism. Spores are produced in very large numbers on the under side of the fruit caps of *Marasmius plicatus,* and they germinate with comparatively little difficulty. But the fruit caps are produced only under exceptionally favorable conditions, and there is no evidence of any increase of the fungus following the production of the spores in a given locality. In the mushroom group as a whole reproduction by spores seems to be of relatively little importance. On the whole, it seems probable that spores have a comparatively unimportant part to play in the propagation of the sugar cane *Marasmius.*

To a much greater extent than among higher plants, detached portions of the vegetative part of fungi can reproduce the plant. Bits of the mycelium of the root fungus of sugar cane grow and spread rapidly under favorable conditions. A small amount on the butt of a planted stalk may be the means of infecting one or more stools of the plant cane to which it gives rise. It is thus by the planting of infected stalks that the root fungus is most efficiently spread from field to field, and continued from year to year.

During the season of 1907 three field tests on two plantations in West Baton Rouge Parish were made to determine what part the planting of affected canes play in the continuance and spread of the fungus. The results were as follows:

Experiment	Rows	Seed Cane	Percentage Affected Stools	Percentage Affected Stalks
A	1	Affected whole stalks	73	30
A	2	Sound whole stalks	23	17
B	1-3	Affected whole stalks	85	63
B	4-6	Sound whole stalks	63	35
C	1-4	Affected whole stalks	89	61
C	5	Affected lower halves	100	91
C	6-10	Sound whole stalks	1.7	0.7

It is noticeable that the amount of fungus was constantly greater when affected stalks were used for planting, and was greatest for the row planted with the lower halves only, that is, with the part having the fungus most abundant. At the outset nothing was known regarding the amount of the fungus that might be present in the soil. In experiments A and B it was later very evident from the showing of the control rows and of the fields in question generally, that the soil was everywhere thoroughly infested with the fungus. This accounts for the rather high percentages for the rows planted with sound cane in these two tests. In experiment C there was very little evidence of the presence of the root fungus in any part of the field, and the control rows remained almost free from it. The cane used for the general planting in all three fields was from the plant crop and showed an unusually small percentage of stalks with adhering shuck.

A third means of distribution is by the persistence of the mycelium of the fungus in the decaying roots and other cane trash of the fields. A certain amount of such infected material must of necessity remain over from one season to the next and can become the source of infection for the new crop of cane. The root fungus similarly protected can withstand lower winter temperatures than can the cane. Young cultures were killed by an exposure of six hours to temperature ranging from 3 degrees to 14 degrees Fahrenheit, but survived an exposure of 45 minutes to the same range. Exposure to 32 degrees for six hours was not fatal. Growth took place slowly during a prolonged exposure to 50 degrees Fahrenheit, and most rapidly at about 85 degrees Fahrenheit. Young cultures survived exposure of 45 minutes to 122 degrees Fahrenheit, but were killed by exposure to the same temperature for one and one-quarter hours.

Further data are necessary before statements can be made regarding the period of persistence of the fungus in the soil when not planted in cane.

OCCURRENCE IN LOUISIANA.

The root disease of sugar cane has existed for a long time in Louisiana and is now distributed rather evenly through all the cane-growing districts of the State. Fructifications which have rendered the identity certain, have been collected from six parishes, and canes with matted leaf sheaths of characteristic appearance have been seen from as many more parishes. The percentage of infestation varies much with local conditions, there being often more noticeable differences in this regard between different parts of the same plantation than between the plantations themselves. It is a usual thing to find the purple plant cane affected to the extent of 5 to 8 per cent of the stalks, and purple first-year stubble 12 to 18 per cent. In the case of D 74, 1 to 3 per cent and 4 to 8 per cent are usual figures for the corresponding crops. Some of the worst fields seen have been in purple cane, and have had 90 or 95 per cent of the stalks infected.

These figures are not to be taken as representing in any way the loss due to the root disease. Affected canes, although usually small and light, are by no means a total loss. And the figures do not take into account the gaps in stands and the reduction in number of canes per stool, that make up the large loss from root disease.

It has been very generally observed that D 74 shows a less degree of infestation than purple. The following figures, which are based on counts from comparable plantings, give indication that the seedling canes D 74 and D 95 are less infected than the two most common home varieties when grown under the same conditions:

FIRST YEAR STUBBLE.

	Fall Plant.	*Spring Plant.*
Striped	34.3%	30.6%
Purple	26.6%	22.9%
D 95	22.2%	16.7%
D 74	14.4%	15.0%

Each percentage represents the average condition of nine rows. The cane used for spring planting was windrowed from the lot from which that used for fall planting was taken.

From another planting during a different season the following figures have been deduced:

SECOND YEAR STUBBLE.

	Fall Plant.
Striped	27.9%
Purple	20.2%
D 95	15.3%
D 74	12.5%

Whether this difference is due to a greater natural resistance in the new canes, such has been reported for the Yellow Caledonia variety in the Hawaiian Islands, or whether it is merely the result of the newness of the varieties, which, on account of great care in the handling of them, have been up to this time less exposed to infection by the root fungus, are questions that can be definitely answered only after further investigation. Nothing is known regarding the relative amounts of infestation of the seed cane used in the above-mentioned plantings.

MODE OF ATTACK.

The two ways in which the root disease fungus works injury to the sugar cane are by destroying the roots, and by smothering the germinating buds. In each case the effect may be produced in an indirect and mechanical way, or more directly by the parasitic attacks of the fungus.

When a number of leaf sheaths are firmly cemented about a portion of the stalk, there is a very serious obstruction to the proper growth of eyes and roots from the nodes involved. It is frequently the case that before such canes are cut, roots are protruded in large numbers from the enveloped nodes above ground; these grow to the length of a fraction of an inch and die, their tips becoming black and shrunken, and their tissues dry and spongy. A microscopic examination shows generally the mycelium of the root fungus within their tissues (Fig. 6). When cuttings from such badly enveloped canes are subjected to conditions suitable for the development of roots and shoots, it is found that in number and vigor of both, such cuttings fall far below sound cuttings, and appreciably below cuttings of the same lot from which the adhering shuck had been removed before planting.

The fungus has the power to attack roots at any period of their growth. When it is brought in contact with a root either naturally or artificially, there can be seen at the first a reddening of the surface of the root at the place of contact; this is followed by a brown discoloration of the area, and later by slow disintegration. A microscopical examination in the early stages shows an abnormal condition of the cells of the affected area, but does not show any penetration of the fungus even into the outermost cells. A little later the fungus is found to have invaded these cells, and by degrees it extends to remoter portions of the root, its advance being always preceded by the death of the cells just ahead. This killing of the cells is in all probability brought about through the agency of substances excreted by the fungus.

The parasitism manifested in these attacks is of a low and weak sort. Unlike more accomplished parasites, which can penetrate and draw sustenance from the living tissues of the host without destroying them, the root fungus of sugar cane must kill the tissues before it can invade or feed upon them. In living upon such tissues only after they are dead, it shows nothing more than a saprophytic mode of living; but the ability which it has to kill lifts it a little above the rank of saprophyte and entitles it to be regarded as a weak parasite.

The abundant presence of the fungus in the adhering leaf sheaths of affected cuttings places it in a situation most convenient for attacking the roots as they are put forth. The growth upward of the fungus on the outer and lower leaf parts of the developing shoot gives it a similar situation with respect to the roots subsequently developed from the underground nodes of the daughter plant. Its persistence on such underground parts of the plant cane keeps it in a position to attack most readily and at an early stage the growing parts of the stubble crop.

CONDITIONS FAVORING ATTACK.

There is a constant struggle for supremacy between a parasite and its host. Those host plants that possess greatest powers of resistance are affected slightly or not at all; less resistant plants succumb readily. In the case under consideration, the balance seems very even between the root fungus and the cane plant. When all conditions are conducive to vigorous, thrifty growth of the cane, the fungus, though present about the stool, has little or no effect upon it. Anything that disturbs such an optimum condition of the cane and weakens it, may be the occasion of more successful attacks by the fungus and greater consequent destructiveness. Cane requires careful cultivation for its best development; the root fungus is a wild plant, a low organism having the ability to withstand and to thrive under a far wider range of conditions than its host. In this way the fungus enjoys an initial natural advantage in the struggle.

Some of the more important conditions that tend to lessen the vigor of the cane and its powers of resistance, and so to increase the chances for the fungus to work injury, are these:

1. *Slowness of germination and early growth.* It is a matter of common observation that fall plant cane and more especially stubble cane have a larger percentage of infection than spring plant cane. This condition probably arises from a long continuance under ground of the fall planted cane and the the stubble stocks before active growth begins, during much of which time the root fungus grows, becoming so well established upon the cane parts as to readily infect the young plants; and infection is further favored by the slow and intermittent character of the growth at first from these parts. It is further

likely that the canes from a stubble crop so generally used for fall planting, have a higher initial infection than the seed cane used in spring planting; this would account for a certain difference in the degree of infestation of the resulting crops.

2. *Improper cultural procedures.* It goes without saying that cane is below its best when proper care has not been taken in the preparation of the soil for planting, and in all subsequent operations until the crop is laid by. The point to be emphasized in the present connection is that the unhealthy, weakened condition of the cane that is the direct result of poor cultivation, is itself a predisposing factor for the destructive attacks of the root fungus. Especially serious are these when cane in its earlier and more susceptible stages of growth has its powers of resistance weakened by improper or inadequate cultivation.

3. *Unsuitable soil.* The root fungus through its ability to subsist upon decaying plant parts, may be found rather abundantly in soil too thin for the proper growing of cane. The under developed and struggling cane plants in such unfavorable situations may fall an easy prey to the root fungus.

4. *Bad drainage.* It is commonly the case that heavy, poorly drained soils show very large proportions of the canes infested with the root fungus. The wetness of such lands makes them especially suitable for the growth of the fungus; and the lack of proper drainage prevents the best development of the cane, with a resulting increase in infection.

5. *Unfavorable seasonal conditions.* Drouth conditions are those under which the worst effects of the root disease are brought about. These effects are not due to any spread of infection to new stools, but to the rapid and pronounced deterioration of stools already infected, the stubble crops being affected far more extensively than the plant crop. The stunting and dying of the stools in such periods of drouth follow directly from the insufficiency of their root systems; and it has been pointed out that the root disease fungus, by attacks upon the young roots, may and does seriously reduce the root systems of the affected stools. In a favorable season those stools, with a slight reduction in root system, show little or no falling off, and even those with a considerable reduction survive, with a loss, however,

in number and size of canes. But when conditions are such as to render it difficult or impossible for stools with full root systems to secure the proper amount of water and food materials, those stools at all lacking in roots suffer appreciably; and those greatly lacking in this regard die.

6. *The stubble crop.* The root disease seldom causes great loss in any but the stubble crops. Some of the causes of this have been indicated. On the one hand, the root fungus has had a chance during the interval between the taking off of the plant crop and the sprouting of the stubble canes, to become thoroughly established in the trash and decaying parts about the stumps. On the other hand, the roots and shoots begin their growth under very disadvantageous conditions which result in large measure from the impossibility of thoroughly working the soil immediately around the stumps.

In addition to causing the death of stubble cane during its period of growth and the general stunting of affected stools that survive, the root fungus probably plays an important part in killing entirely during the winter months some of the stools so as to leave noticeable gaps from the very first in stubble crops when the plant crops had a perfect stand. It is believed that this effect is brought about by the complete killing and smothering of roots and eyes, rather than by disintegration of the nodes themselves through the direct agency of the root disease fungus.

PREVENTIVE MEASURES.

Procedures for the control of the root disease are at once suggested by an understanding of the modes of invasion and dissemination of the fungus, that is, its causative agent. Briefly summarized, such procedures must follow the lines of proper cultivation and the use of sound seed cane. Preventive measures must be relied upon, not remedial ones.

1. *Careful cultivation.* It is necessary but to mention in this connection proper tillage, active and thorough cultivation, especially of the stubble crops, good drainage, and rotation of crops. When it is a matter of combatting root disease, all possible care must be taken that the environmental conditions of the cane plant are such that it may maintain a healthy and vigorous condition especially during the critical period of early

growth. Effective resistance at this time goes far toward securing freedom from the devastating effects of the root disease.

2. *Selection and disinfection of seed cane.* It has been seen that the planting of infected canes, that is, those having the leaves matted at the base, not only introduces the fungus throughout the field, but does it in such way that the plant stools readily become infected. To avoid this is important. Sound cane only should be used in planting. Non-infected seed cane may be secured in two ways: (1) By careful inspection of cane for planting with the rejection of all suspected ones. (2) By thorough disinfection with some good fungicide, such as Bordeaux mixture. Of these two methods the former is less expensive, but is probably less effective, since there is always a chance that some very slightly infected stalks will be overlooked. Both methods are tedious and costly when carried out on a plantation scale. But by setting apart a tract of superior land that is free from root fungus infestation, for growing seed cane, and by careful selection and perhaps disinfection of the seed cane for the general planting can be obtained at a minimum expense.

2. *Resistant varieties.* All the evidence now at hand shows greater freedom from root disease in the case of the seedling varieties D 74 and D 95. Exact statements regarding a possible natural resistance to the disease possessed by these must be deferred until tests now in progress yield results. In any event, there is a certain amount of present benefit as regards freedom from root disease to be derived from the planting of the new canes.

4. *Destruction of infected trash.* The mycelium of the root disease fungus lives over from season to season in the dead and decaying parts of affected canes that are left in the field. These are an important source of infection to the new crop. Burning over the fields destroys much infected trash; and this practice, as well as the removal as thoroughly as possible of old cane stumps, tends to hold the root disease in check.

5. *Resting land from cane.* In some instances the root disease fungus has become so thoroughly distributed and so well established in particular fields as to render the operation of the foregoing measures inadequate for a proper control of the

disease. The application of fungicidal substances to the soil over large tracts has generally proved unsuccessful for the eradication of fungi which live in the soil and attack the underground parts of plants. It is not likely that any degree of success would attend the use of such substances in the present case. Since there is no evidence that the sugar cane *Marasmius* has any other plant than sugar cane for a host plant, it is reasonable to suppose that the amount of its persisting in the soil can be materially reduced by keeping cane off the land for several seasons. The usual rotation covering a three or four-year period, doubtless has its beneficial effect in lessening the amount of root fungus in the soil. Unfortunately, data are too incomplete for anything to be said about the length of time necessary for the complete eradication by this method of the fungus from a badly infested field. Frequent plowing for the purpose of thoroughly exposing the soil to the sun and drying it would doubtless destroy much of the fungus. The stubble crops are not only the ones in which the greatest losses occur, but they are the ones that best serve as nurseries for the fungus. When the plant crop on any area is so badly affected by the root disease as to make the margin of profit dangerously small, it should be borne in mind that the stubble crop will show even worse effects of the disease, and that the keeping of the land in cane for a second season under the circumstances will increase the chance for serious infection of subsequent cane crops; and the matter of leaving the stubble or planting an entirely different crop should be decided in the light of these considerations.

Success is attending the use in other countries of the measures mentioned. A recent letter from Dr. H. C. Prinsen-Geerligs conveys the information that it is now difficult to find specimens of the root fungus in Java. The system practiced there of quick rotation with the elimination of the stubble crops, and the great care taken with the material for planting, have no doubt played an important part in bringing about the results. The work of control in the Hawaiian Islands has been too recently undertaken for definite results. Published accounts of late date from the British West Indies indicate that careful cultivation, rotation of crops, selection of sound seed cane, and the choice of disease resistant varieties, are measures that are being used there with an encouraging degree of success.

TECHNICAL DESCRIPTION.

Specimens of the Louisiana root disease fungus have been submitted to Dr. W. G. Farlow, who has very kindly examined them, and has expressed the opinion that they are to be regarded provisionally *Marasmius plicatus*, Wakker. Since it has not been possible to make comparison with a specimen of this species, it has seemed advisable to include the following technical description of the Louisiana fungus:

Pileus 12-35 mm., usually 15-20 mm., across; flesh thin, somewhat pliant; convex with the margins somewhat incurved, then expanded and more or less depressed; even, glabrous, at length rugose and wrinkled; whitish, becoming tawny when dry. Lamellae adnexed, sometimes joined behind and leaving the stem; distant, unequal, often connected by veins, rather broad, thin, even, white. Stipe stuffed, then hollow, fibrous inside, with a cartilaginous cuticle, almost glabrous above, base tuberculose and flocculose; whitish, 5-20x0.8—1.8 mm., usually 12x1 mm. Spores white, ovate, and with a prolongation at the attached end, 6.8x5.6 mm. Mycelium whitish to cream color, flocculose becoming cemented by gelatinous envelopes, sparingly septate, with clamp connections, about 3 mm. in diameter. On leaves, roots and decaying parts of sugar cane, having the habit of a weak parasite. Greparious, scattered or somewhat cespitose.

The sporophores are shown in Figs. 2 and 3; the spores in Fig. 7, and the mycelium in Fig. 8.

EFFECT OF TEMPERATURE ON GROWTH.

In testing the effect of different temperatures upon the mycelium of *Marasmius plicatus*, cultures in test tubes on slightly acidulated potato agar were used. The exposures were made when growth was well started, that is, 20 to 30 hours after inoculation; but in a few instances cultures four or five days old were tested without showing important difference from the younger cultures. The more important results are given below:

Exposure.	Temperature degrees C.	Growth during exposure.	Growth after exposure.
6 hours	—10 to —16		None. Dead
½ hour	—10 to —16		Fair
6 hours	0		Good
5 days	3 to 5	None	Good
5 days	11	Slight	Excellent
5 days	20	Good	Excellent
5 days	25	Excellent	Excellent
5 days	30	Excellent	Excellent
5 days	35	Good	Excellent
5 days	41 to 42	None	None. Dead
6 hours	45		Good
2½ hours	50		None. dead
1¼ hours	50		Fair
¾ hour	52		Fair

RESISTANCE TO DRYING.

When plugs of sugar cane from test tube cultures nine to ten months old and thoroughly air dry were placed in moist chambers, growth was secured in about one-fourth of the trials. The fungus was also recovered from the mycelial growth well up on the sides of flask cultures of a like age, with steamed rice as culture medium. A cane stalk that had remained for ten months in the rather dry laboratory atmosphere yielded a good growth from detached portions of the matted leaf sheaths.

CONDITIONS FAVORING FRUITING.

Before fructifications were found in the field various attempts were made to secure them from pure cultures. For this purpose six strains of fungus were grown in flasks on a wide range of nutrient media and under varied conditions of temperature and moisture. The mycelial growth was very much the same in all cases, and nothing suggestive of sporophore formation was apparent. The first fructification seen was found in June on a cane plant grown in a tub and naturally affected with root disease from the infected cutting planted. The lower part of this stalk had been covered with a glass cylinder pushed into the ground and plugged at the top with cotton, so that the atmospheric conditions about the sporophore were rather moist. On August 4th, after several days of cloudy weather, with intermittent showers, the fruit-bodies were found in great abundance on several plantations examined, and their constant association with the mycelium cementing the leaf sheaths was definitely determined. They were also found on detached pieces of decaying cane that were permeated with typical *Marasmius* mycelium, and on exposed broken roots of still living cane stools, the roots also being filled with the mycelium. From this time until the last of September it was possible to find fruit bodies whenever conditions were sufficiently wet in the cane rows, and not during drier periods. The fruit caps were usually found on the bases of the stalks within an inch or two of the surface of the ground, where the leaf sheaths were quite moist. Careful search during October and November, when moisture conditions seemed favorable, was unsuccessful. It would seem probable that rather high degrees of moisture and warmth are required for fruiting.

INOCULATION EXPERIMENTS.

In the first series, which included about twenty plants, cane-cuttings were sprouted in damp moss, and transferred to tubs filled with clean sand. Mycelium of three strains from pure cultures was used for inoculation material, and was placed in contact with the young sprouts at the time of setting out. The plants were watered with Sachs' nutrient solution. These inoculations were successful in three-fourths of the trials.

The second series was with young cane in the field several weeks before it began to joint, and while the lowermost leaf sheaths were apparently in a living condition. The inoculation material was from pure cultures of two strains, and was applied variously above the ground, below the ground, to uninjured sheaths, to incised sheaths, and in some cases with protection from drying by means of paraffined pasteboard cylinders, and in other cases without such protection. After two weeks, during which period weather conditions happened to be most favorable, every inoculation had taken.

After fructifications had been secured, successful inoculations were made with pure cultures from spores and from the stipe, as well as from the sheath-cementing mycelium.

In the laboratory roots were infected frequently and in various stages of development by growing them in moist chambers and placing portions of agar cultures in contact with them. Natural infection of the roots was secured readily by keeping nodes that were well covered by the adhering leaf sheaths in moist chambers. Young leaves inserted in test tubes containing an abundant growth of the fungus were readily infected.

In the case of each marked external discoloration and pathological change in the outermost cells followed quickly, and only after these had taken place could the fungus threads be found pervading the tissues. The infected areas afterward spread rapidly. The mycelium had an intracellular disposition. The first foothold on roots was usually at the point of emergence from the stalk where a slight projection of dead stalk tissue furnished a good feeding ground for the fungus (Fig. 6). Next in frequency under natural conditions was entrance from the root cap. Here it was noticeable that not the growing point, but that part of the root tip covered by the proximal portion of the root cap was the point of first entrance. In the roots the cortex was the region most extensively infested, and only occasionally were fungus threads found in the fibro-vascular bundles.

In the case of leaves, entrance was not generally through the stomates, but usually by a direct penetration of epidermal cells. Both parenchymatous and vascular elements were permeated.

All indications point to the conclusion that this species of *Marasmius* gains entrance only after the outside cells of the attacked portion of the cane plant have been killed; and this killing is accomplished presumably through some physiological activity of the fungus.

Infection experiments were not made with other plants than sugar cane. In only one case was the fungus seen growing out of doors on any other plant: in a field where there was abundant evidence of considerable soil infection, two fruit-bodies were found on a dead piece of Bermuda grass, and the leaf sheath was cemented to the stem by the mycelium in characteristic fashion.

LITERATURE.

The following references are to the more important published accounts of the root disease of sugar cane:

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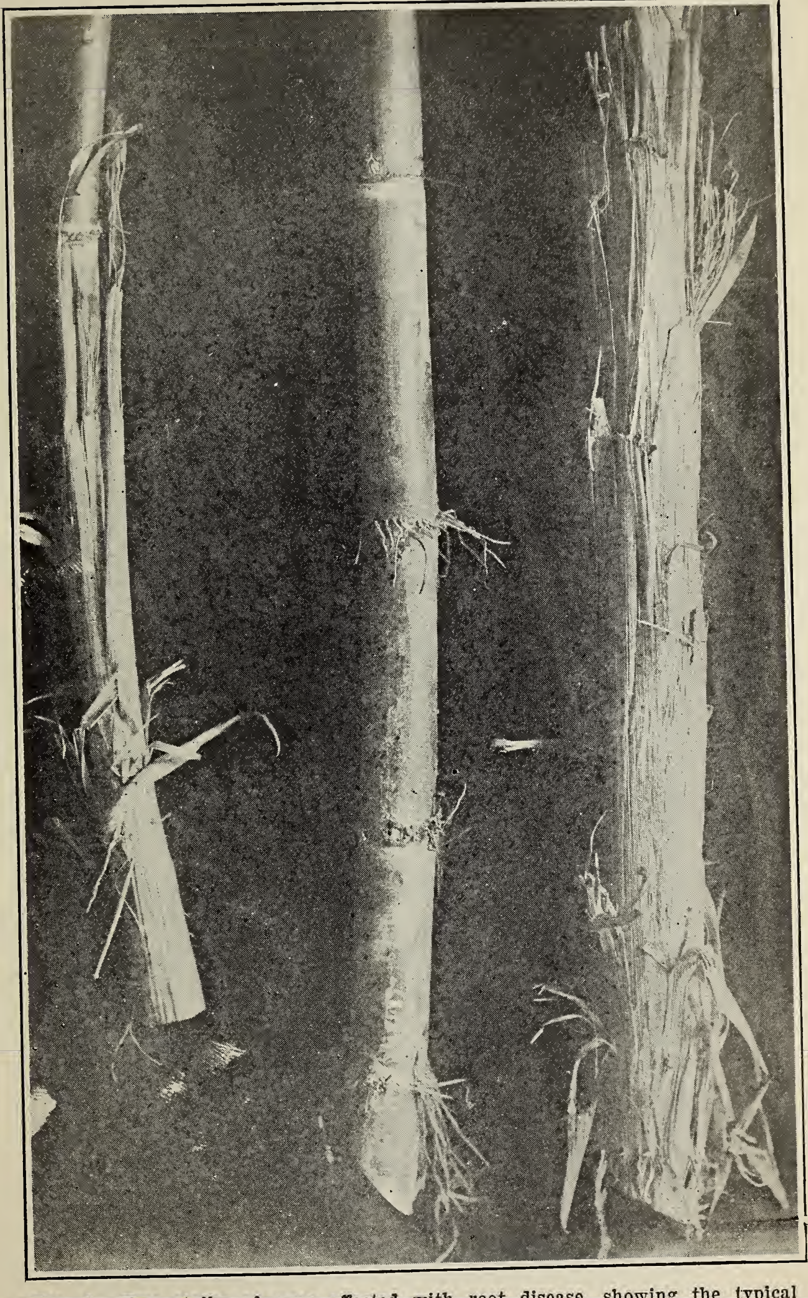

FIG. 1.—Two stalks of cane affected with root disease. showing the typical cementing of the shuck. The middle unaffected stalk is for comparison.

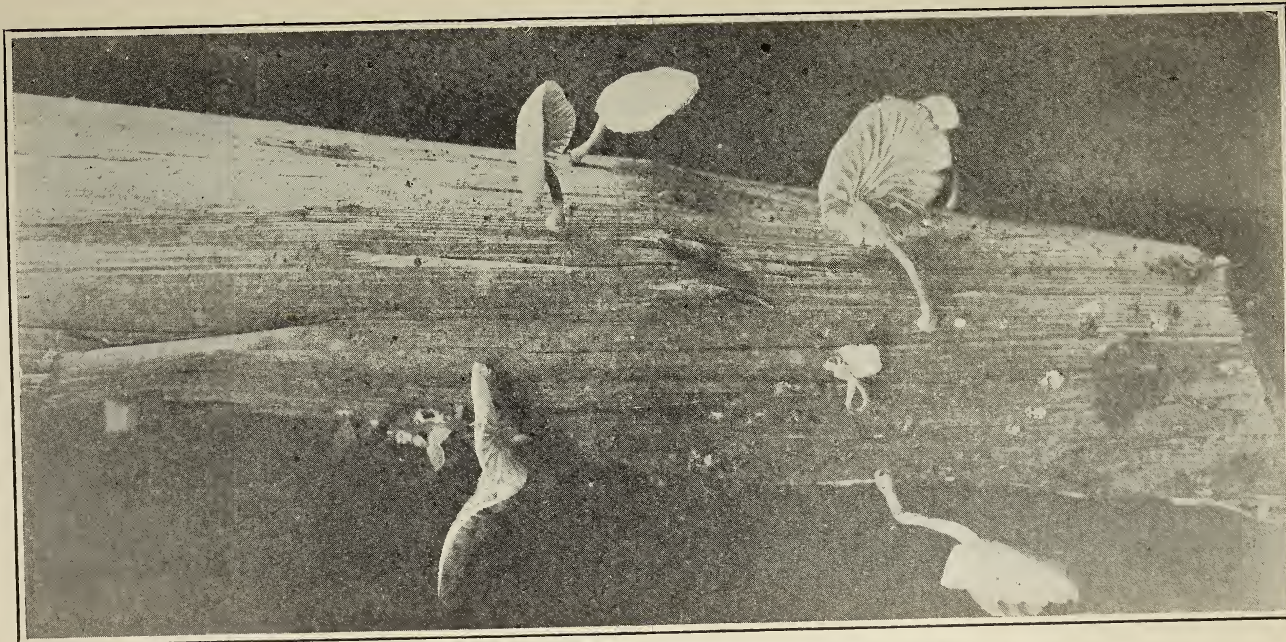

FIG. 2.—Fructifications of the fungus causing root disease of sugar cane in Louisiana, and supposed to be *Marasmius plicatus*, Wakker. x 4-5.

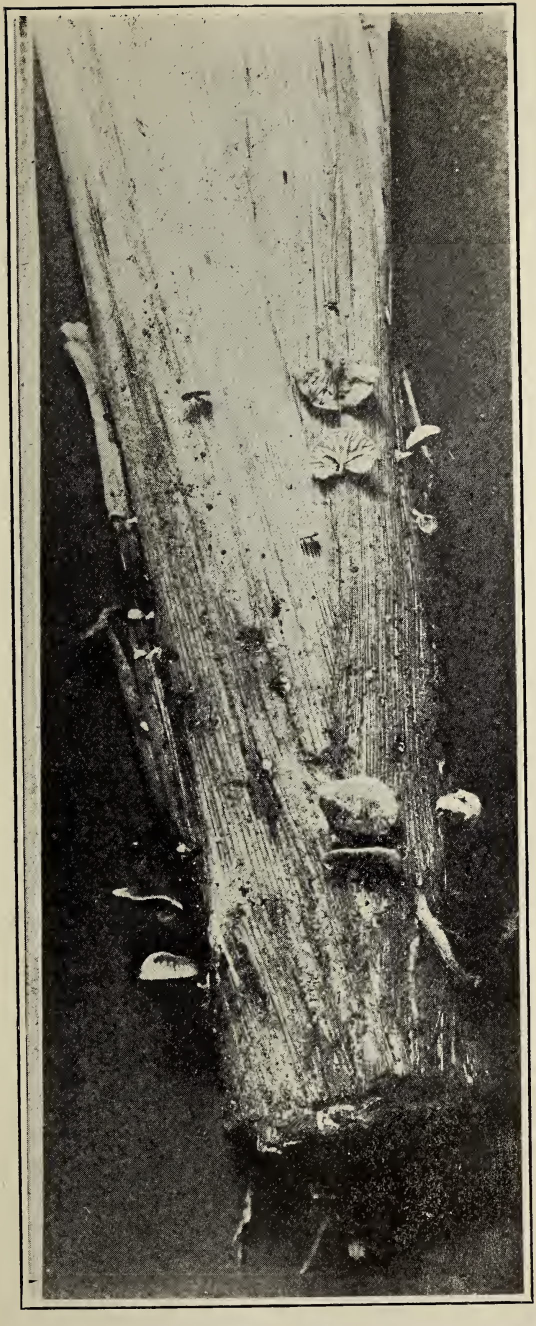

FIG. 3.—Fructifications of the fungus causing root disease of sugar cane in Louisiana, and supposed to be *Marasmius plicatus*, Wakker. x 4-5.

FIG. 4.—Stock of cane affected with root disease. The white mycelium is growing vigorously on the young shoots, and in the case of the long root extending to the right, white tufts of mycelium protrude from the **breaks in the bark.**

FIG. 5.—Portion of a field of stubble cane where the loss is very great. The few canes left in the affected area show the root fungus very prominently.

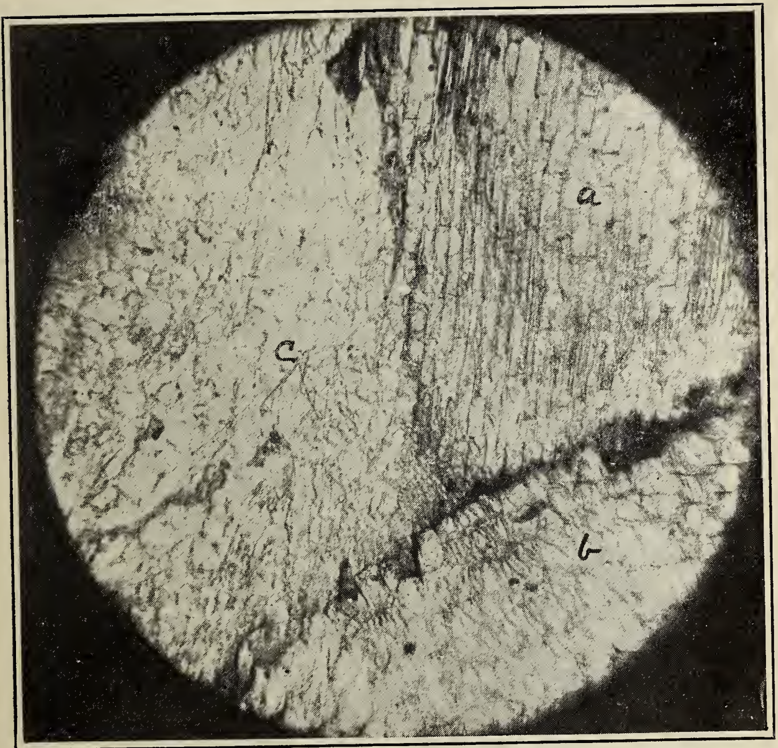

FIG. 6.—Microphotograph showing invasion of sugar cane root by the threads of the root disease fungus. *a* is a portion of the main root cut longitudinally; *b* is a root branch cut transversely; and *c* is a portion of disintegrated tissue from the mother stalk at the point of emergence, which is full of fungus threads. These are seen entering the cells of *a* and of *b*.

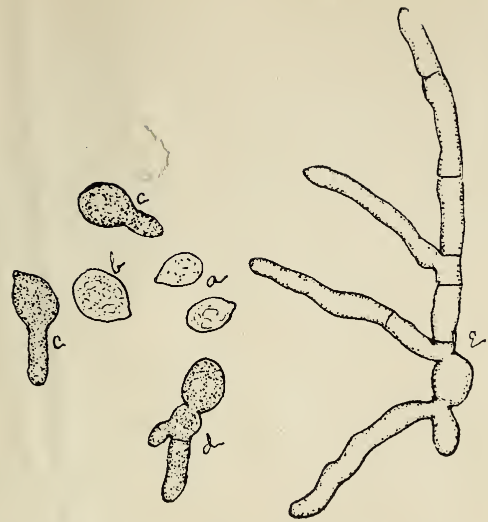

FIG. 7.—*a*. Spores of *Marasmius plicatus*, normal size. *b*. Spore swollen previous to germination. *c*. Spores in early stages of germination. *d* and *e*. Later stages of germination. x 1,000.

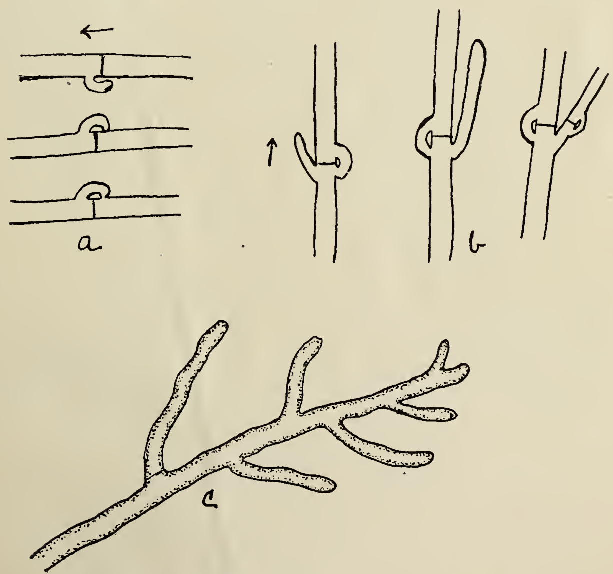

FIG. 8.—*a*. Mycelium of *Maramius plicatus*, showing stages in the formation of the clamp connections. *b*. Mycelium showing branches. *c*. An actively growing tip of the mycelium. x 1,000.